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Toxicity and DNA damage in tobacco and potato plants growing on soil polluted with heavy metals

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Abstract

Heterezygous tobacco (*Nicotiana tabacum* var. *xanthi*) and potato (*Solanum tuberosum* var. Korela) plants were cultivated on soil from the site Střimice which is highly polluted with heavy metals and on nonpolluted soil from the recreational site Jezeří, both in North Bohemia, Czech Republic. The total content, the content of bioavailable, easily mobile, and potentially mobile components of heavy metals (Cd, Cu, Pb, and Zn) in the tested soils, and the accumulation of these metals in the above-ground biomass and roots of tested plants were analyzed by flame atomic absorption spectrometry or flameless atomic absorption spectrometry. The average tobacco leaf area and potato plant height were significantly reduced in plants growing on the polluted soil. We have measured the DNA damage in nuclei of leaves of both plant species using the Comet assay. A small but significant increase in DNA damage was noted in plants growing on the polluted soil versus controls. As the tobacco and potato plants with increased DNA damage were severely injured (inhibited growth, distorted leaves), this increase may be associated with necrotic or apoptotic DNA fragmentation. No increase in the frequency of somatic mutation was detected in tobacco plants growing on the polluted soil. Thus, the polluted soil probably induced toxic but not genotoxic effects on tobacco and potato plants.

Keywords: Cd; Comet assay; Cu; Ethyl methanesulfonate; Pb; Single cell gel electrophoresis; Somatic mutations; Zn

1. Introduction

Plants are exposed to various types of environmental xenobiotics, either deliberately as in the case of agricultural pesticides and plant growth regulators or accidentally as compounds present in polluted air, soil, or water. For most crops growing in polluted areas or treated with agricultural chemicals, no genotoxicity assays are available. We have studied the possibility of using the Comet assay (Singh et al., 1988; Tice et al., 2000; Collins, 2004) as a method for detecting induced DNA damage in plants.

In the work presented here we have cultivated tobacco and potato plants on soil polluted with heavy metals (site Střimice, polluted soil) and on soil from a recreational area (Jezeří, nonpolluted soil) for 4, 8, or 12 weeks and (1) assessed the toxic effects of the soil samples by measuring the tobacco leaf area and potato plant height, (2) evaluated induced DNA damage in leaf nuclei by the Comet assay, (3) scored the frequency of somatic mutations in tobacco plants, and (4) determined by inductively coupled plasma optical emission spectrometry the amount of Cd, Cu, Pb, and Zn in the soil samples and in the tobacco and potato above-ground biomass and roots. For our studies we have selected potatoes (*Solanum tuberosum*) as an important crop plant and the model heterozygous tobacco (*Nicotianan tabacum* var. *xanthi*) where induced somatic mutations can be scored.

2. Material and methods

2.1. Chemicals

Ethyl methanesulfonate (EMS; CAS No. 62-50-0), maleic hydrazide (MH; CAS No. 123-33-1), reagents for electrophoresis, and normal and low-melting-point agarose were purchased from Sigma Chemical Co.

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(St. Louis, MO); reagents for the determination of the contents of heavy metals in the soil and in the tested plants were purchased from Analytika and Lach-Ner Ltd. (Czech Republic).

2.2. Sample collection

Soil samples were taken from two sites: from the site Střimice (a sink of tailing rocks and mine spoils) and from the recreational area Jezeří, both in North Bohemia. The soil samples were air-dried at 20 °C, ground in a mortar, and passed through a 2-mm plastic sieve.

2.3. Soil analysis

2.3.1. Total content of Cd, Cu, Pb, and Zn

Aliquots (0.5 g) of the air-dried and sieved soil samples were decomposed in borosilicate glass test tubes at 400 °C for 10 h in a mixture of oxidizing gases ($O_2+O_3+NO_x$) in an Apion Dry Mode Mineralizer (Tessek, CZ) (Miholová et al., 1993). The ash was dissolved in a mixture of 65% nitric and 38% hydrofluoric acid (2:1), evaporated to dryness at 160 °C, dissolved in 37% HCl and 65% HNO_3 (3:1), and kept in glass tubes until measurement (Száková et al., 1999). Aliquots of the certified reference material RM 7001 Light Sandy Soil were mineralized under the same conditions for quality assurance of the analytical data. In the reference material containing Cd = 0.32, Cu = 30.8, Pb = 43.8, and Zn = 120 mg kg^{-1} soil, total amounts of Cd = 0.38, Cu = 33.0, Pb = 41.8, and Zn = 119 mg kg^{-1} soil were detected.

2.3.2. Content of bioavailable, easily mobile, and potentially mobile components of Cd, Cu, Pb, and Zn

Three fractions of the heavy metals in the soil were determined: (a) bioavailable; aliquots (10 g) of dried soil samples were extracted with 1 mol L^{-1} solution of NH_4NO_3 in ratio 1:2.5 (w/v) for 2 h at 20 °C (Pruess et al., 1991), (b) easily mobile; aliquots (1 g) of dried soil samples were extracted with 0.43 mol L^{-1} solution of CH_3COOH in ratio 1:40 (w/v) for 5 h at 20 °C (Quevauviller et al., 1993), and (c) potentially mobile; aliquots (3 g) of dried soil samples were extracted with 2 mol L^{-1} solution of HNO_3 in ratio 1:10 (w/v) for 6 h at 20 °C (Borůvka et al., 1997).

The soil extracts were centrifuged (Hettich Universal 30 RF) at 3000 rpm (460*g*) for 10 min and the supernatants were kept at 6 °C.

The metal content of the soil digests and extracts in the case of high element content were determined by flame atomic absorption spectrometry (F-AAS; Varian SpectrAA-300). The method of flameless atomic absorption spectrometry (ETAAS; Varian SpectrAA-400 with graphite tube atomizer GTA-96) was applied when the element concentration was under detection limit of flame AAS (see Table 1).

2.3.3. Analysis of the content of Cd, Cu, Pb, and Zn in tobacco and potato plants

For the determination of the accumulation of metals in tobacco and potato plants, aliquots (1 g) of the dried and powdered roots or above-ground biomass were decomposed in 50-mL quartz-glass beakers at 500 °C for 16 h on a hot plate and in muffle furnace with a stepwise increase of the ashing temperature (Mader et al., 1998). The ash was then dissolved in 20 mL of 1.5% solution of HNO_3. The content of elements in the plant digests was determined by inductively coupled plasma optical emission spectrometry with axial plasma configuration (ICP-AES; Varian VistaPro, Australia), equipped with an autosampler SPS-5, at spectral lines λ = 226.5 nm for Cd, λ = 327.4 nm for Cu, λ = 220.4 nm for Pb, and λ = 206.2 nm for Zn.

Aliquots of the certified reference materials V-10 Hay (for tobacco plants) and BCR 281 rye grass (for potato plants) were mineralized under the same conditions for quality assurance of the analytical data. In the reference material V-10 Hay containing Cd = 0.12, Cu = 9.65, Pb = 2.38, and Zn = 31.5 mg kg^{-1} dry mass, total amounts of Cd = 0.13, Cu = 8.93, Pb = 1.55, and Zn = 29.0 mg kg^{-1} dry mass were detected. In the reference material BCR 281 containing Cd = 0.04, Cu = 9.25, Pb = 1.35, and Zn = 24.0 mg kg^{-1} dry mass, total amounts of Cd = 0.05, Cu = 9.10, Pb = 1.38, and Zn = 21.80 mg kg^{-1} dry mass were detected.

2.4. Bioassays

2.4.1. Tobacco growth, treatment conditions, and measurement of the leaf area and the frequency of somatic mutations

Seeds of the double heterozygous *N. tabacum* var. *xanthi* (a_1^+/a_1; a_2^+/a_2) plants (Dulieu and Dalebroux, 1975) were germinated in pots with garden soil and at the stage of three or four true leaves transferred to 26 plastic pots with the tested soils. The plants in the containers were cultivated in a growth chamber at 22–26 °C with a 16-h per day photoperiod.

After 4 weeks of cultivation, the area of 3 leaves per plant (mostly the 5th, 6th, and 7th leaves) and after 8 weeks (mostly the 8th, 9th and 10th leaves), numbered from the bottom, were measured by a planimeter and expressed in cm^2. Three main types of mutagenic events were scored on the greenish-yellow leaves of the heterozygous tobacco plants cultivated on the tested soils: (1) dark green, (2) yellow, and (3) green/yellow twin sectors (Dulieu and Dalebroux, 1975). The somatic mutations were scored under a stereomicroscope.

Table 1
Comparison of total content and content of bioavailable, mobile, and potentially mobile fractions of Cd, Cu, Pb, and Zn after various extraction solutions of the soil from the polluted site Střimice and the recreational site Jezeří (average contents with standard error)

Site	Metal	Total content (mg kg^{-1} soil)	Extraction solutions		
			NH_4NO_3 (1 mol L^{-1}) (mg kg^{-1} soil)	CH_3COOH (0.43 mol L^{-1}) (mg kg^{-1} soil)	HNO_3 (2 mol L^{-1}) (mg kg^{-1} soil)
Střimice	Cd	11.4 ± 0.18[a]	0.63 ± 0.01[b]	1.00 ± 0.02[b]	5.51 ± 0.13[b]
Jezeří		0.14 ± 0.006[b]	0.08 ± 0.03[b]	0.05 ± 0.003[b]	0.13 ± 0.001[b]
Střimice	Cu	556 ± 28[a]	1.68 ± 0.09[a]	36.4 ± 0.69[a]	317 ± 6[a]
Jezeří		19 ± 0.3[a]	0.12 ± 0.02[b]	0.26 ± 0.01[b]	7.49 ± 0.42[b]
Střimice	Pb	12,190 ± 220[a]	649 ± 14[a]	1854 ± 15[a]	9103 ± 219[a]
Jezeří		47.6 ± 0.5[a]	1.4 ± 0.02[a]	1.68 ± 0.07[a]	41.3 ± 1.86[a]
Střimice	Zn	1,292 ± 1[a]	19.5 ± 0.4[a]	90.2 ± 1.39[a]	667 ± 12[a]
Jezeří		132 ± 6[a]	11.9 ± 0.3[a]	12.9 ± 0.86[a]	34.8 ± 2.23[a]

[a]Flame atomic absorption spectrometry.
[b]Atomic absorption spectrometry with graphite tube atomizer.

2.4.2. Potato growth, treatment conditions, and measurement of plant height

Isolated buds of potato (*S. tuberosum* var. Korela) were cultivated in containers with soil from the site Střimice (polluted soil) and from the recreational site Jezeří (nonpolluted soil). In each experiment, 100 containers with polluted and 100 containers with nonpolluted soil were used. The plants in the containers were cultivated in a greenhouse at 22–28 °C with a 16-h per day photoperiod and, after 4, 8, and 12 weeks of cultivation, the height of the plants was measured.

2.4.3. DNA damage studies

After cultivating tobacco and potato plants on polluted and nonpolluted soil, excised leaves of the plants were placed in a 60-mm petri dish kept on ice and spread with 250 μL of cold 400 mM Tris buffer, pH 7.5. Using a fresh razor blade, the leaves were gently sliced, and the isolated nuclei were collected in the buffer. All operations were conducted under dim or yellow light. The preparation of agarose microscope slides with isolated nuclei was earlier described (Gichner et al., 2004). The slides were placed in a horizontal gel electrophoresis tank containing freshly prepared cold electrophoresis buffer (1 mM Na_2EDTA and 300 mM NaOH, pH > 13). For tobacco plants, the nuclei were incubated for 10 min to allow the DNA to unwind prior to electrophoresis at 0.72 V cm^{-1} (26 V, 300 mA) for 25 min at 4 °C. For potato plants, the unwinding time was 5 min and the electrophoresis time 15 min. After electrophoresis, the slides were stained with 80 μL ethidium bromide (20 μg mL^{-1}) for 5 min, dipped in ice-cold water to remove the excess ethidium bromide, and covered with a coverslip. For each slide, 25 randomly chosen nuclei were analyzed using a fluorescence microscope with an excitation filter of BP 546/10 nm and a barrier filter of 590 nm. A computerized image analysis system (Komet version 3.1; Kinetic Imaging Ltd., Liverpool, UK) was employed. The tail moment (TM; the amount of DNA in the Comet tail multiplied by the tail length, divided by 100, and expressed in micrometers) was used as the measure of DNA damage. For DNA damage studies, five leaves were taken from different plants of each treatment variant, and from each leaf two SCGE slides were prepared. In total, 250 nuclei were analyzed per variant, if not otherwise stated.

To demonstrate the efficiency of the Comet assay in tobacco and potato plants, the monofuctional alkylating agent ethyl methanesulfonate was applied. Roots of tobacco seedlings of the four to five true-leaf stage and 1-month-rooted cuttings of potato plants were immersed in glass vials containing 22 mL of defined concentrations of EMS for 24 h at 26 °C. Then isolated nuclei from leaves were processed for the Comet assay.

2.5. Statistical analysis

Data were analyzed using the statistical and graphical functions of SigmaPlot 8.01 and SigmaStat 3.0 (SPSS Inc., Chicago, IL, USA). The median tail moment values were used in a one-way analysis of variance test. If a significant F value of $P \leqslant 0.05$ was obtained, a Dunnett's multiple comparison versus the control group analysis was conducted.

3. Results

3.1. Total content of Cd, Cu, Pb, and Zn in the soil

The total content of heavy metals (Table 1) determined by atomic absorption spectrometry in the polluted soil from the site Střimice (Cd = 11, Cu = 556, Pb = 12,190, and Zn = 1292 mg kg^{-1} soil) is significantly ($P \leqslant 0.001$) higher than their content in the soil from the site Jezeří (Cd = 0.14, Cu = 19, Pb = 48, and Zn = 132 mg kg^{-1} soil). The content of Pb in the soil sample from site Střimice is 254-fold higher than that in the sample from site Jezeří, and for Cd the content is 82-fold higher in the polluted soil.

The content of bioavailable, easily mobile, and potentially mobile fractions for all four studied elements in the soil is an important factor for their uptake by plants. The bioavailable fraction of the metals in the soil obtained by extraction with 1 mol L^{-1} NH_4NO_3 releases most of the plant-available heavy metal fraction. It represents 5.5% of the total content of Cd, 0.3% of Cu, 5.3% of Pb, and 1.5% of Zn for the site Střimice and 57.2% of Cd, 0.6% of Cu 2.9% of Pb, and 9% of Zn for site Jezeří. The easily mobile fraction, obtained by extraction with 0.43 mol L^{-1} CH_3COOH, releases exchangeable water and acid-soluble (e.g., specifically adsorbed on soil oxides) metal fractions (Miller et al., 1986; Száková et al., 2000). This fraction represents 8.8% of Cd, 6.5% of Cu, 15.2% of Pb, and 6.9% of Zn from the soil of site Střimice and 39.1% of Cd, 1.4% of Cu, 3.6% of Pb, and 9.8% of Zn from the site Jezeří. The highest amount of metals was extracted from the soil by 2 mol L^{-1} HNO_3, representing potentially mobile soil elements, i.e., the metal fraction immobile under stable soil conditions but mobile if the soil parameters (e.g., soil pH or sorption capacity) are dramatically changed. Nitric acid is able to dissolve most soil elements with the exception of the immobile element fraction bound tightly into the silicate matrix of the soil (Száková et al., 2000).

3.2. Uptake of Cd, Cu, Pb, and Zn by tobacco and potato plants

Table 2 summarizes the total content of heavy metals in the above-ground biomass and the roots of the tobacco plants cultivated for 4 and 8 weeks and that of potato plants cultivated for 4, 8, and 12 weeks on the polluted and nonpolluted soils. The uptake of heavy metals in both crop plants growing on the contaminated soil is higher than that growing on the nonpolluted soil. For example, the uptake of Pb in the above-ground biomass of tobacco plants growing on soil from the site Střimice was 162 compared to 2.9 mg kg^{-1} dry mass in plants growing on the soil from the site Jezeří.

Comparison of the accumulation and translocation of the studied metals within the plants shows that the uptake of the metals by the roots is higher than the uptake in the above-ground biomass. For example, the uptake of Pb in the above-ground biomass of tobacco plants growing on soil from the site Střimice was 162 compared to 1200 mg kg^{-1} dry mass in the roots. The only exception was the uptake of Cd by tobacco plants, where on the same soil the above-ground biomass contained 35.9 compared to 16.8 mg kg^{-1} dry mass in the roots. The sum of bioavailable (after extraction with NH_4NO_3) and easily mobile (after extraction with of CH_3COOH) was Pb = 2503 mg kg^{-1} soil; the total amount of Pb in tobacco plants was 1362 and that in potato plants was 1621 mg kg^{-1} dry mass.

Table 2
Comparison of Cd, Cu, Pb, and Zn accumulation in above-ground biomass and roots of tobacco (*Nicotiana tabacum* var. xanthi) and potato (*Solanum tuberosum* var. Korela) plants after cultivation on soil from the polluted site Střimice and the recreational site Jezeří

Site	Metal	Tobacco (*Nicotiana tabacum*)		Potato (*Solanum tuberosum*)	
		Above-ground biomass ($mg\,kg^{-1}$ dry mass)	Roots ($mg\,kg^{-1}$ dry mass)	Above-ground biomass ($mg\,kg^{-1}$ dry mass)	Roots ($mg\,kg^{-1}$ dry mass)
Střimice	Cd	35.9	16.8	6.3	17.4
Jezeří		1.5	0.4	0.7	1.1
Střimice	Cu	29.6	61.3	24.4	155
Jezeří		23.8	5.0	15.0	24.9
Střimice	Pb	162	1200	51.2	1570
Jezeří		2.9	3.1	6.9	21.6
Střímice	Zn	1533	3130	1616	5644
Jezeří		73.2	43.7	326	704

3.3. Experiments with tobacco plants

3.3.1. Treatment with ethyl methanesulfonate

Fig. 1 illustrates the DNA-damaging effects of EMS on tobacco plants, expressed by the average median tail moment values. With increasing concentrations of EMS, the TM ($\pm$SE) values of leaf nuclei significantly ($P \leqslant 0.001$) increased from $5.0 \pm 1.5\,\mu m$ (negative control) to $68.0 \pm 2.3\,\mu m$ (8 mM EMS).

3.3.2. Toxic, mutagenic, and DNA-damaging effects in tobacco plants cultivated on soil with high levels of metals

After 4 weeks of cultivation of tobacco plants on the soil from the polluted site Střimice the average leaf area of $20.0\,cm^2$ was significantly lower ($P \leqslant 0.001$) than the average leaf area of $45.8\,cm^2$ of tobacco plants cultivated on soil from the recreational site Jezeří (Table 3). While after 8 weeks of cultivation on the nonpolluted soil the averaged leaf area significantly ($P \leqslant 0.001$) increased to $64.1\,cm^2$, tobacco plants growing on the polluted soil had leaves with average area of only $13.6\,cm^2$, thus even lower than after 4 weeks of cultivation. This decrease is explained by the strong inhibitory effects of the polluted soil on the roots followed by an inhibition of the growth of above-ground biomass and smaller leaves.

The increased frequency of somatic mutation would be evidence of genotoxic effects of the polluted soil. After 4 weeks of cultivation of the heterozygous tobacco plants, the frequency of mutations in tobacco plants growing on the polluted soil was very low (0.40 per leaf scored) and did not significantly ($P = 0.914$) differ from the frequency (0.46 per leaf scored) in plants growing on the nonpolluted soil. After 8 weeks of cultivation, the frequency of induced mutations of tobacco plants growing on the nonpolluted soil was also very low (0.73 per leaf); nevertheless it was significantly ($P \leqslant 0.001$) higher than the frequency of mutations in tobacco plants growing on polluted soil (0.32 per leaf). This difference can be explained by the very small size of the leaves of plants growing on polluted soil which prevents the detection of small mutated sectors. For comparison, the herbicide and plant growth regulator maleic hydrazide induced in tobacco 37.8 mutations per leaf (Table 3).

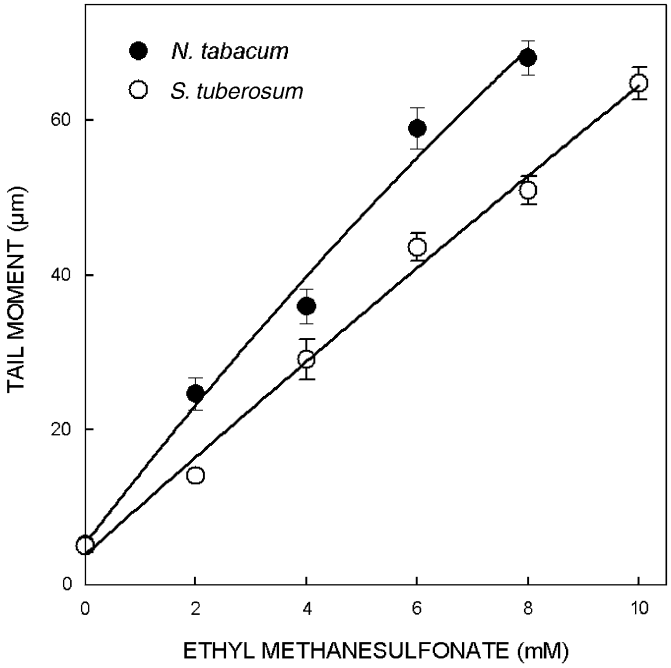

Fig. 1. Dose–response curves of the DNA damage in nuclei of *Nicotiana tabacum* var. *xanthi* and *Solanum tuberosum* var. Korela leaves as a function of ethyl methanesulfonate treatment for 24 h at 26 °C. The error bars represent the standard error of the average medians. At least 100 nuclei were evaluated per treatment.

The results of monitoring DNA damage in leaf nuclei of tobacco plants growing on the soil from the site Střimice (polluted soil) and on the soil from the site Jezeří (nonpolluted soil) are presented in the last column of Table 3. Although the DNA damage after 4 and 8 weeks

Table 3
Leaf area, frequency of somatic mutations, and DNA damage in tobacco plants (*Nicotiana tabacum* var. xanthi) cultivated for 4 and 8 weeks on soil from the polluted site Střimice and the recreational site Jezeří

Locality	Cultivation (weeks)	Leaves scored	Averaged leaf area ($cm^2 \pm SE$)	Mutant sectors per leaf ± SE	Tail moment (μm ± SE)
Střimice	4	74	20.0 ± 0.8	0.40 ± 0.1	9.0 ± 0.4
Jezeří		78	45.8 ± 1.7	0.46 ± 0.1	4.4 ± 0.6
Střimice	8	51	13.6 ± 0.7	0.32 ± 0.1	10.7 ± 0.7
Jezeří		76	64.1 ± 1.2	0.53 ± 0.1	6.0 ± 0.5
MH[a]	4	18	36.7 ± 2.1	37.8 ± 3.2	—
EMS[b]	2	3	—	—	45.2 ± 2.6

[a]MH. Tobacco plant cultivated on sand wetted three times a week with 0.05 mM maleic hydrazide dissolved in 50% Hoagland solution.
[b]EMS. Tobacco plants treated with 0.04 mM ethyl methanesulfonate dissolved in 50% Hoagland solution. The solutions were changed every second day.

Table 4
Plant height and DNA damage as measured by the Comet assay in potato plants (*Solanum tuberosum* var. Korela) cultivated for 4, 8, and 12 weeks on soil from the polluted site Střimice and the recreational site Jezeří

Locality	Cultivation (weeks)	Plants measured	Averaged plant height (cm ± SE)	Tail moment (μm ± SE)
Střimice	4	78	6.1 ± 1.5	5.6 ± 0.9
Jezeří		96	13.7 ± 1.8	2.4 ± 0.3
Střimice	8	63	10.7 ± 1.3	6.1 ± 0.8
Jezeří		96	17.1 ± 1.9	2.5 ± 0.3
Střimice	12	46	12.9 ± 1.2	7.1 ± 0.5
Jezeří		94	18.0 ± 1.2	2.4 ± 0.2

cultivation of tobacco plants on the polluted soil was comparatively low (TM = 9.0 and 10.7 μm, respectively) these values were significantly ($P \leqslant 0.001$) higher than the values of TM in leaf nuclei isolated from tobacco plants cultivated on nonpolluted soil (TM = 4.4 and 6.0 μm, respectively).

3.4. Experiments with potato plants

3.4.1. Treatment with ethyl methanesufonate

The DNA-damaging effects of EMS on potato plants, expressed by the average median TM, are illustrated in Fig. 1. With increasing concentrations of EMS, the TM (±SE) values of leaf nuclei significantly ($P \leqslant 0.001$) increased from 5.0 ± 0.8 μm (negative control) to 64.8 ± 2.1 μm (10 mM EMS).

3.4.2. Toxic and DNA-damaging effects in potato plants cultivated on soil with high levels of metals

After 4 weeks of cultivation of potato plants on the soil from the polluted site Střimice, the average plant height 6.1 cm was significantly lower ($P \leqslant 0.001$) than the plant height 13.7 cm of potato plants cultivated on soil from the recreational site Jezeří (Table 4). The height of potato plants cultivated 8 and 12 weeks on the polluted soil (10.7 and 12.9 cm, respectively) was also significantly ($P \leqslant 0.001$) lower than the height of control plants (17.1 and 18.0 cm, respectively). At the start of the experiment, 100 potato plants were cultivated on each of tested soils. The number of plants that survived the cultivation on the polluted soil (46) was much lower than the number of plants on control, nonpolluted soil (94). These data clearly demonstrate the toxic effects of the polluted soil on potato plant growth.

The results of monitoring DNA damage in leaf nuclei of potato plants growing on the soil from the site Jezeří (nonpolluted soil) and on soil from the site Střimice (polluted soil) are presented in the last column of Table 4. Although the DNA damage after 4, 8, and 12 weeks of cultivation of potato plants on the polluted soil was comparatively low (TM = 5.6, 6.1, and 7.1 μm, respectively) these values were significantly ($P \leqslant 0.001$) higher than the values of TM in leaf nuclei isolated from potato plants cultivated on nonpolluted soil (2.5, 2.5, and 2.4 μm, respectively).

4. Discussion

Heavy metals are released into the environment by power stations, heating systems, waste incinerators, metal-working industries, and many other sources. Their accumulation in soils can become dangerous to all kinds of organisms, including plants. However, there is an increasing recognition that elevated contaminant levels in themselves are not necessarily indicative of actually

occurring adverse effects. Identification and quantification of metal fractions associated with individual soil components, together with physicochemical (pH, clay and oxides content, etc.) and microbial and enzymatic parameters of the soil, can lead to a better characterization of the bioavailability and bioaccessibility of heavy metals and to a better understanding of their possible adverse toxic and genotoxic effects (Peijnenburg and Jager, 2003; Száková et al., 1999).

The most important factor for elucidating the possible adverse effects of the metals is their actual accumulation in the plants. The tobacco plants cultivated on the polluted soil Střimice accumulated especially high amounts of Zn = 4663 and Pb = 1362 mg kg^{-1} dry mass in the above-ground biomass and roots. These values do not, however, correlate with the total amount of both elements in the soil (Zn = 1292 and Pb = 12,190 mg kg^{-1} soil).

Even though the toxic effects of heavy metals in plants have been studied over many years, inconsistent results in their genotoxic properties have been obtained. Both positive and negative results have been reported, depending on the test material and the evaluated metal (Panda and Panda, 2002; White and Claxton, 2004). In tobacco plants, Cd^{2+} induced DNA damage measured by the Comet assay but no somatic mutations and homologous recombination (Gichner et al., 2004). In *Tradescantia* pollen mother cells and in *Allium cepa* and *Vicia faba* root tip cells, Pb^{2+}, Cd^{2+}, and Zn^{2+} caused a dose-dependent increase of micronuclei, while Cu^{2+} gave negative responses (Steinkellner et al., 1998). Cultivating *Tradescantia* plants on soil from industrial areas in the vicinity of metal smelters in Austria with high contents of Zn, Pb, Cu, and Cd resulted in a 11-to-15 fold increase in the frequency of micronuclei over the control values (Knasmüller et al., 1989). No data on the genotoxicity of metals in potato plants are available.

The Comet assay is a powerful genetic assay for the analysis of DNA damage in eukaryotic cells (Tice et al., 2000; Collins, 2004). The alkaline version of the Comet assay can quantitatively measure DNA damage, including single-strand breaks, double-strand breaks, alkali-labile sites (primarily apurinic and apyrimidine sites), incomplete excision repair sites, and DNA cross-links. Although this technique has been primarily applied to animal cells, the incorporation of the Comet assay with plant tissues (Koppen and Verschaeve, 1996; Gichner and Plewa, 1998; Menke et al., 2001) significantly extends the utility of plants in basic and applied studies in environmental mutagenesis.

By the selective uptake or accumulation of heavy metals, the growth of tobacco plants (expressed by the average leaf area) and of potato plants (expressed by the plant height) on soil with high contents of Cd, Cu, Pb, and Zn is strongly inhibited compared to the growth of plants on nonpolluted soil. The tobacco and potato plants with increased DNA damage versus controls were severely injured (inhibited growth, distorted and brownish leaves). Many of the Comet images from these distorted leaves were of the "hedgehog" type (large fan-like tail and small heads). This may be associated with necrotic or apoptotic DNA fragmentation (Gichner et al., 2005). No increase in the frequency of somatic mutation was detected in tobacco plants growing on the polluted soil. Thus, the polluted soil with heavy metals probably induced toxic but not genotoxic effects on tobacco and potato plants.

5. Conclusions

The levels of Cd, Cu, Pb, and Zn in soil from the highly polluted site Střimice seriously exceeded the levels of these metals in nonpolluted soil from the control recreational site Jezeří, both in North Bohemia, Czech Republic. In heterezygous tobacco (*N. tabacum* var. xanthi) and potato (*S. tuberosum* var. Korela) plants growing on the polluted soil, high toxic effects (inhibited growth, distorted leaves) and a small but significant increase in DNA damage were noted versus controls.

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